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(54) Title: NOVEL COMPOUNDS

(57) Abstract

A soluble protein having IL4 and/or IL13 antagonist or partial antagonist activity comprises an IL4 mutant or variant fused to at least one human immunoglobulin constant domain or fragment thereof.

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NOVEL COMPOUNDS

The present invention relates to antagonists of human interleukin 4 (IL4) and/or human interleukin 13 (IL13) for the treatment of conditions resulting from undesirable actions of IL4 and/or IL13 such as certain IgE mediated allergic diseases.

5 T cell mediated autoimmune conditions and inappropriate immune responses to infectious agents.

Interleukins are secreted peptide mediators of the immune response. Each of the known interleukins has many effects on the development, activation, proliferation and differentiation of cells of the immune system. IL4 has a physiological role in such functions, but can also contribute to the pathogenesis of disease. In particular 10 IL4 is associated with the pathway of B lymphocyte development that leads to the generation of IgE antibodies that are the hallmark of allergic diseases such as extrinsic asthma, rhinitis, allergic conjunctivitis, atopic dermatitis and anaphylaxis. IL4 can also act as a general growth and differentiation factor for T lymphocytes that 15 may contribute to tissue damage in certain autoimmune conditions such as insulin dependent diabetes, multiple sclerosis and rheumatoid arthritis and in graft rejection. IL4 can also suppress the generation of cell-mediated responses required for the control of infectious disease. Antagonism of the effect of IL4 on T or B lymphocytes can therefore be expected to have beneficial effects on such diseases. IL13 has been 20 recently identified and shares similarity in many of the biological properties of IL4 (Minty, A. *et al* (1993), Nature 362, 248-250) including some aspect(s) of receptor structure/function (Aversa, G. *et al* (1993), J. Exp. Med. 178, 2213-2218).

Human IL4 consists of a single polypeptide chain of 129 amino acids with 2 possible N-glycosylation sites and 6 cysteines involved in 3 disulphide bridges (Le, H.V. *et. al.*, (1988), J. Biol. Chem. 263, 10817-10823). The amino acid sequence of 25 IL4 and the positions of these disulphide bridges are known (Carr, C. *et al.*, (1991) Biochemistry 30, 1515-1523).

	10	
30	HIS-LYS-CYS-ASP-ILE-THR-LEU-GLN-GLU-ILE-ILE-LYS-THR-LEU-ASN-	
20	30	
35	SER-LEU-THR-GLU-GLN-LYS-THR-LEU-CYS-THR-GLU-LEU-THR-VAL-THR-	
40	40	
40	60	
50	CYS-ARG-ALA-ALA-THR-VAL-LEU-ARG-GLN-PHE-TYR-SER-HIS-HIS-GLU-	
80	70	
80	LYS-ASP-THR-ARG-CYS-LEU-GLY-ALA-THR-ALA-GLN-GLN-PHE-HIS-ARG-	
90	90	

HIS-LYS-GLN-LEU-I_{LE}-ARG-PHE-LEU-LYS-ARG-LEU-ASP-ARG-ASN-LEU-
100
TRP-GLY-LEU-ALA-GLY-LEU-ASN-SER-CYS-PRO-VAL-LYS-GLU-ALA-ASN-
5
110
GLN-SER-THR-LEU-GLU-ASN-PHE-LEU-GLU-ARG-LEU-LYS-THR-I_{LE}-MET-
120
10
129
ARG-GLU-LYS-TYR-SER-LYS-CYS-SER-SER

The disulphide bridges are between residues 3 and 127, 24 and 65, and 46 and 99. The molecular weight of IL4 varies with the extent of glycosylation from 15KDa (no glycosylation) to 60KDa or more (hyperglycosylated IL4).

15 The DNA sequence for human IL4 has also been described by Yokota, T. *et. al.*, P.N.A.S. 1986 83 5894-5898.

WO 93/10235 describes certain mutants of IL4 which are IL4 antagonists or partial antagonists.

20 EP-A-0 464 533 discloses fusion proteins comprising various portions of the constant region of immunoglobulin molecules together with another human protein or part thereof.

The present invention provides a soluble protein having IL4 and/or IL13 antagonist or partial antagonist activity, comprising an IL4 mutant or variant fused to at least one human immunoglobulin constant domain or fragment thereof.

25 The term "mutant or variant" encompasses any molecule such as a truncated or other derivative of the IL4 protein which retains the ability to antagonise IL4 and/or IL13 following internal administration to a human. Such other derivatives can be prepared by the addition, deletion, substitution, or rearrangement of amino acids or by chemical modifications thereof.

30 DNA polymers which encode mutants or variants of IL4 may be prepared by site-directed mutagenesis of the cDNA which codes for IL4 by conventional methods such as those described by G. Winter *et al* in Nature 1982, 299, 756-758 or by Zoller and Smith 1982; Nucl. Acids Res., 10, 6487-6500, or deletion mutagenesis such as described by Chan and Smith in Nucl. Acids Res., 1984, 12, 2407-2419 or by G. Winter *et al* in Biochem. Soc. Trans., 1984; 12, 224-225 or polymerase chain reaction such as described by Mikaelian and Sergeant in Nucleic Acids Research, 1992, 20, 376.

35 As used herein, "having IL4 and/or IL13 antagonist or partial antagonist activity" means that, in the assay described by Spits *et al* (J. Immunology 139, 1142 (1987)), IL4-stimulated T cell proliferation is inhibited in a dose-dependent manner.

40 Suitable IL4 mutants are disclosed in WO 93/10235, wherein at least one amino acid, naturally occurring in wild type IL4 at any one of positions 120 to 128

inclusive, is replaced by a different natural amino acid. In particular, the tyrosine naturally occurring at position 124 may be replaced by a different natural amino acid, such as glycine or, more preferably, aspartic acid.

- The immunoglobulin may be of any subclass (IgG, IgM, IgA, IgE), but is preferably IgG, such as IgG1, IgG3 or IgG4. The said constant domain(s) or fragment thereof may be derived from the heavy or light chain or both. The invention encompasses mutations in the immunoglobulin component which eliminate undesirable properties of the native immunoglobulin, such as Fc receptor binding and/or introduce desirable properties such as stability. For example, Angal S., King D.J., Bodmer M.W., Turner A., Lawson A.D.G., Roberts G., Pedley B. and Adair R., Molecular Immunology vol30pp105-108, 1993, describe an IgG4 molecule where residue 241 (Kabat numbering) is altered from serine to proline. This change increases the serum half-life of the IgG4 molecule. Canfield S.M. and Morrison S.L., Journal of Experimental Medicine vol173pp1483-1491, describe the alteration of residue 248 (Kabat numbering) from leucine to glutamate in IgG3 and from glutamate to leucine in mouse IgG2b. Substitution of leucine for glutamate in the former decreases the affinity of the immunoglobulin molecule concerned for the Fc_Y RI receptor, and substitution of glutamate for leucine in the latter increases the affinity. EP0307434 discloses various mutations including an L to E mutation at residue 248 (Kabat numbering) in IgG.
- The constant domain(s) or fragment thereof is preferably the whole or a substantial part of the constant region of the heavy chain of human IgG, most preferably IgG4. In one aspect the IgG component consists of the CH2 and CH3 domains and the hinge region of IgG1 including cysteine residues contributing to inter-heavy chain disulphide bonding, for example residues 11 and 14 of the IgG1 hinge region (Frangione B. and Milstein C., Nature vol216pp939-941, 1967). Preferably the IgG1 component consists of amino acids corresponding to residues 1-4 and 6-15 of the hinge, 1-110 of CH2 and 1-107 of CH3 of IgG1 described by Ellison J., Berson B. and Hood L. E., Nucleic Acids Research vol10, pp4071-4079, 1982.
- Residue 5 of the hinge is changed from cysteine in the published IgG1 sequence to alanine by alteration of TGT to GCC in the nucleotide sequence. In another aspect the IgG component is derived from IgG4, comprising the CH2 and CH3 domains and the hinge region including cysteine residues contributing to inter-heavy chain disulphide bonding, for example residues 8 and 11 of the IgG4 hinge region (Pinck J.R. and Milstein C., Nature vol216pp941-942, 1967). Preferably the IgG4 component consists of amino acids corresponding to residues 1-12 of the hinge, 1-110 of CH2 and 1-107 of CH3 of IgG4 described by Ellison J., Buxbaum J. and Hood L.. DNA vol1pp11-18, 1981. In one example of a suitable mutation in IgG4, residue 10

of the hinge (residue 241, Kabat numbering) is altered from serine (S) in the wild type to proline (P) and residue 5 of CH2 (residue 248, Kabat numbering) is altered from leucine (L) in the wild type to glutamate (E).

- 5 Fusion of the IL4 mutant or variant to the Ig constant domain or fragment is by C-terminus of one component to N-terminus of the other. Preferably the IL4 mutant or variant is fused via its C-terminus to the N-terminus of the Ig constant domain or fragment.

In a preferred aspect, the amino acid sequence of the fusion protein of the invention is represented by SEQ ID No:4, SEQ ID No:7 or SEQ ID No:10.

- 10 In a further aspect, the invention provides a process for preparing a compound according to the invention which process comprises expressing DNA encoding said compound in a recombinant host cell and recovering the product.

The DNA polymer comprising a nucleotide sequence that encodes the compound also forms part of the invention.

- 15 In a preferred aspect the DNA polymer comprises or consists of the sequence of SEQ ID No:3, SEQ ID No:6 or SEQ ID No:9.

The process of the invention may be performed by conventional recombinant techniques such as described in Maniatis *et. al.*, Molecular Cloning - A Laboratory Manual; Cold Spring Harbor, 1982 and DNA Cloning vols I, II and III (D.M. Glover ed., IRL Press Ltd).

- 20 In particular, the process may comprise the steps of:
- i) preparing a replicable expression vector capable, in a host cell, of expressing a DNA polymer comprising a nucleotide sequence that encodes said compound;
 - ii) transforming a host cell with said vector;
 - 25 iii) culturing said transformed host cell under conditions permitting expression of said DNA polymer to produce said compound; and
 - iv) recovering said compound.

30 The invention also provides a process for preparing the DNA polymer by the condensation of appropriate mono-, di- or oligomeric nucleotide units.

- 35 The preparation may be carried out chemically, enzymatically, or by a combination of the two methods, *in vitro* or *in vivo* as appropriate. Thus, the DNA polymer may be prepared by the enzymatic ligation of appropriate DNA fragments, by conventional methods such as those described by D. M. Roberts *et al* in Biochemistry 1985, 24, 5090-5098.

The DNA fragments may be obtained by digestion of DNA containing the required sequences of nucleotides with appropriate restriction enzymes, by chemical

synthesis, by enzymatic polymerisation on DNA or RNA templates, or by a combination of these methods.

Digestion with restriction enzymes may be performed in an appropriate buffer at a temperature of 20°-70°C, generally in a volume of 50µl or less with 0.1-10µg

5 DNA.

Enzymatic polymerisation of DNA may be carried out *in vitro* using a DNA polymerase such as DNA polymerase I (Klenow fragment) in an appropriate buffer containing the nucleoside triphosphates dATP, dCTP, dGTP and dTTP as required at a temperature of 10°-37°C, generally in a volume of 50µl or less.

10 Enzymatic ligation of DNA fragments may be carried out using a DNA ligase such as T4 DNA ligase in an appropriate buffer at a temperature of 4°C to ambient, generally in a volume of 50µl or less.

15 The chemical synthesis of the DNA polymer or fragments may be carried out by conventional phosphotriester, phosphite or phosphoramidite chemistry, using solid phase techniques such as those described in 'Chemical and Enzymatic Synthesis of Gene Fragments - A Laboratory Manual' (ed. H.G. Gassen and A. Lang), Verlag Chemie, Weinheim (1982), or in other scientific publications, for example M.J. Gait, H.W.D. Matthes, M. Singh, B.S. Sproat, and R.C. Titmas, Nucleic Acids Research, 1982, 10, 6243; B.S. Sproat and W. Bannwarth, Tetrahedron Letters, 1983, 24, 5771; 20 M.D. Matteucci and M.H. Caruthers, Tetrahedron Letters, 1980, 21, 719; M.D. Matteucci and M.H. Caruthers, Journal of the American Chemical Society, 1981, 103, 3185; S.P. Adams *et al.*, Journal of the American Chemical Society, 1983, 105, 661; N.D. Sinha, J. Biernat, J. McMannus, and H. Koester, Nucleic Acids Research, 1984, 12, 4539; and H.W.D. Matthes *et al.*, EMBO Journal, 1984, 3, 801. Preferably an automated DNA synthesizer is employed.

25 The DNA polymer is preferably prepared by ligating two or more DNA molecules which together comprise a DNA sequence encoding the compound. A particular process in accordance with the invention comprises ligating a first DNA molecule encoding a said IL4 mutant or variant and a second DNA molecule encoding a said immunoglobulin domain or fragment thereof.

30 The DNA molecules may be obtained by the digestion with suitable restriction enzymes of vectors carrying the required coding sequences or by use of polymerase chain reaction technology.

35 The precise structure of the DNA molecules and the way in which they are obtained depends upon the structure of the desired product. The design of a suitable strategy for the construction of the DNA molecule coding for the compound is a routine matter for the skilled worker in the art.

The expression of the DNA polymer encoding the compound in a recombinant host cell may be carried out by means of a replicable expression vector capable, in the host cell, of expressing the DNA polymer. The expression vector is novel and also forms part of the invention.

5 The replicable expression vector may be prepared in accordance with the invention, by cleaving a vector compatible with the host cell to provide a linear DNA segment having an intact replicon, and combining said linear segment with one or more DNA molecules which, together with said linear segment, encode the compound, under ligating conditions.

10 The ligation of the linear segment and more than one DNA molecule may be carried out simultaneously or sequentially as desired.

Thus, the DNA polymer may be preformed or formed during the construction of the vector, as desired.

15 The choice of vector will be determined in part by the host cell, which may be prokaryotic, such as *E. coli*, or eukaryotic, such as mouse C127, mouse myeloma, chinese hamster ovary or Hela cells, fungi e.g. filamentous fungi or unicellular yeast or an insect cell such as Drosophila. The host cell may also be a transgenic animal. Suitable vectors include plasmids, bacteriophages, cosmids and recombinant viruses derived from, for example, baculoviruses, vaccinia or Semliki Forest virus.

20 The preparation of the replicable expression vector may be carried out conventionally with appropriate enzymes for restriction, polymerisation and ligation of the DNA, by procedures described in, for example, Maniatis *et al.*, cited above. Polymerisation and ligation may be performed as described above for the preparation of the DNA polymer. Digestion with restriction enzymes may be performed in an appropriate buffer at a temperature of 20°-70°C, generally in a volume of 50µl or less with 0.1-10µg DNA.

25 The recombinant host cell is prepared, in accordance with the invention, by transforming a host cell with a replicable expression vector of the invention under transforming conditions. Suitable transforming conditions are conventional and are described in, for example, Maniatis *et al.*, cited above, or "DNA Cloning" Vol. II, D.M. Glover ed., IRL Press Ltd, 1985.

30 The choice of transforming conditions is determined by the host cell. Thus, a bacterial host such as *E. coli* may be treated with a solution of CaCl₂ (Cohen *et al.*, Proc. Nat. Acad. Sci., 1973, 69, 2110) or with a solution comprising a mixture of RbCl, MnCl₂, potassium acetate and glycerol, and then with 3-[N-morpholino]-propane-sulphonic acid, RbCl and glycerol. Mammalian cells in culture may be transformed by calcium co-precipitation of the vector DNA onto the cells.

The invention also extends to a host cell transformed with a replicable expression vector of the invention.

Culturing the transformed host cell under conditions permitting expression of the DNA polymer is carried out conventionally, as described in, for example, 5 Maniatis *et al* and "DNA Cloning" cited above. Thus, preferably the cell is supplied with nutrient and cultured at a temperature below 45°C.

10 The expression product is recovered by conventional methods according to the host cell. Thus, where the host cell is bacterial, such as *E. coli* it may be lysed physically, chemically or enzymatically and the protein product isolated from the resulting lysate. If the product is to be secreted from the bacterial cell it may be recovered from the periplasmic space or the nutrient medium. Where the host cell is 15 mammalian, the product may generally be isolated from the nutrient medium.

15 The DNA polymer may be assembled into vectors designed for isolation of stable transformed mammalian cell lines expressing the product; e.g. bovine papillomavirus vectors or amplified vectors in chinese hamster ovary cells (DNA cloning Vol.II D.M. Glover ed. IRL Press 1985; Kaufman, R.J. *et al.*, Molecular and Cellular Biology 5, 1750-1759, 1985; Pavlakis G.N. and Hamer, D.H., Proceedings of the National Academy of Sciences (USA) 80, 397-401, 1983; Goeddel, D.V. *et al.*, European Patent Application No. 0093619, 1983).

20 Compounds of the present invention have IL4 and/or IL13 antagonist activity and are therefore of potential use in the treatment of conditions resulting from undesirable actions of IL4 and/or IL13 such as IgE mediated allergic diseases and T cell mediated autoimmune conditions or chronic microbial infection.

25 The invention therefore further provides a pharmaceutical composition comprising a compound of the invention and a pharmaceutically acceptable carrier.

30 In use the compound will normally be employed in the form of a pharmaceutical composition in association with a human pharmaceutical carrier, diluent and/or excipient, although the exact form of the composition will depend on the mode of administration. The compound may, for example, be employed in the form of aerosol or nebulisable solution for inhalation or sterile solutions for parenteral administration.

35 The dosage ranges for administration of the compounds of the present invention are those to produce the desired effect on the IL4 and/or IL13 mediated condition, for example whereby IgE antibody mediated symptoms are reduced or progression of the autoimmune disease is halted or reversed. The dosage will generally vary with age, extent or severity of the medical condition and contraindications, if any. The unit dosage can vary from less than 1mg to 300mg, but

typically will be in the region of 1 to 20mg per dose, in one or more doses, such as one to six doses per day, such that the daily dosage is in the range 0.02-40mg/kg.

Compositions suitable for injection may be in the form of solutions, suspensions or emulsions, or dry powders which are dissolved or suspended in a suitable vehicle prior to use.

5 Fluid unit dosage forms are prepared utilising the compound and a pyrogen-free sterile vehicle. The compound, depending on the vehicle and concentration used, can be either dissolved or suspended in the vehicle. Solutions may be used for all forms of parenteral administration, and are particularly used for 10 intravenous infection. In preparing solutions the compound can be dissolved in the vehicle, the solution being made isotonic if necessary by addition of sodium chloride and sterilised by filtration through a sterile filter using aseptic techniques before filling into suitable sterile vials or ampoules and sealing. Alternatively, if solution stability is adequate, the solution in its sealed containers may be sterilised by 15 autoclaving. Advantageously additives such as buffering, solubilising, stabilising, preservative or bactericidal, suspending or emulsifying agents and/or local anaesthetic agents may be dissolved in the vehicle.

Dry powders which are dissolved or suspended in a suitable vehicle prior to use may be prepared by filling pre-sterilised drug substance and other ingredients into 20 a sterile container using aseptic technique in a sterile area. Alternatively the drug and other ingredients may be dissolved in an aqueous vehicle, the solution is sterilised by filtration and distributed into suitable containers using aseptic technique in a sterile area. The product is then freeze dried and the containers are sealed aseptically.

25 Parenteral suspensions, suitable for intramuscular, subcutaneous or intradermal injection, are prepared in substantially the same manner, except that the sterile compound is suspended in the sterile vehicle, instead of being dissolved and sterilisation cannot be accomplished by filtration. The compound may be isolated in a sterile state or alternatively it may be sterilised after isolation, e.g. by gamma irradiation. Advantageously, a suspending agent for example polyvinylpyrrolidone is 30 included in the composition to facilitate uniform distribution of the compound.

Compositions suitable for administration via the respiratory tract include aerosols, nebulisable solutions or microfine powders for insufflation. In the latter case, particle size of less than 50 microns, especially less than 10 microns, is preferred. Such compositions may be made up in a conventional manner and 35 employed in conjunction with conventional administration devices.

In a further aspect there is provided a method of treating conditions resulting from undesirable actions of IL4 and/or IL13 which comprises administering to the sufferer an effective amount of a compound of the invention.

The invention further provides a compound of the invention for use as an active therapeutic substance, in particular for use in treating conditions resulting from undesirable actions of IL4 and/or IL13.

5 The invention also provides the use of a compound of the invention in the manufacture of a medicament for treating conditions resulting from undesirable actions of IL4 and/or IL13.

No unexpected toxicological effects are expected when compounds of the invention are administered in accordance with the present invention.

The following Examples illustrate the invention.

10

Example 1 IL4.Y124D/IgG1 fusion protein

15 The construction of an IL4.Y124D/IgG1 chimeric cDNA, the expression of the corresponding protein in a mammalian expression system and its activity are described.

1. **Construction of DNA coding for fusion protein**

(a) **Construction of IL4.Y124D coding region**

20 A variant of the human IL4 gene, which has been described (Kruse, N, Tony, H-P and Sebald, W. EMBO Journal 11: 3237 [1992]) in which residue 124 in the protein has been mutated from tyrosine in the wild type to aspartic acid, was produced by PCR mutagenesis of the human IL4 cDNA (purchased from British Biotechnology). The IL4.Y124D cDNA was inserted into the expression vector pTR312, using the HindIII and BglII sites, (M J Browne, J E Carey, C G Chapman, A 25 W R Tyrrell, C Entwistle, G M P Lawrence, B Reavy, I Dodd, A Esmail & J H Robinson. Journal of Biological Chemistry 263: 1599, [1988]) to form the plasmid pDB906.

30 To amplify the IL4.Y124D molecule and add convenient restriction sites at each end for subcloning, a PCR reaction was performed using 20ng of the pDB906 plasmid as the substrate. PCR primers were designed to include restriction enzyme sites, flanked by 10-15 nucleotide base pairs to "anchor" the primers at each end. The primer sequences were as follows:

- 1) 5' CGA ACC ACT GAA TTC CCG ATT GCA GAG ATA 3'
35 (includes an EcoRI restriction site, GAATTC)
- 2) 5' CAC AAA GAT CCT TAG GTA CCG CTC GAA CAC TTT GA 3'
(includes a KpnI restriction site, GGTACC)

Primers were used at a final concentration of 5ng/ μ l, and dNTPs were added at a final concentration of 0.2mM in a total reaction volume of 100 μ l. 31 cycles of PCR were performed. Cycles consisted of a denaturation step of 1 minute at 94°C, an annealing step of 1 minute 30 seconds at 50°C, and an elongation step of 1 minute 30 seconds at 72°C. On cycle 1 denaturation was extended to 5 minutes and on the final cycle elongation was extended to 7 minutes. 2.5 units of the Taq polymerase enzyme from Advanced Biotechnologies were used in the PCR reaction. A PCR product of 587bp was produced. This was purified using the Promega "Magic PCR cleanup" kit, and then digested with EcoRI and KpnI in react buffer 4 (all restriction enzymes were obtained from GibcoBRL.), to generate 'sticky ends'. After 4 hours 30 minutes at 37°C, the reaction was heated to 70°C for 10 minutes and then ethanol precipitated. Analysis of the resulting DNA by agarose gel electrophoresis showed the presence of three bands of approximately 570bp, 463bp and 100bp. The 570bp fragment represents the full-length IL4.Y124D variant of IL4 and was present because the digest was incomplete. The two smaller fragments were produced due to the presence of an EcoRI site within the IL4.Y124D cDNA. The 570bp band was purified by the Geneclean™ procedure, and ligated into Bluescript KS+™ which was prepared by digestion with EcoRI and KpnI followed by Geneclean™. A Bluescript KS+/IL4.Y124D recombinant was thus generated. Large amounts of this recombinant DNA were produced using the Promega "Magic Maxiprep" method. The IL4.Y124D insert was excised from the Bluescript recombinant using SmaI and KpnI. 20 μ g recombinant DNA was incubated with 25 units SmaI in react buffer 4, at 30°C overnight. 25 units of KpnI were then added to the digest, which was incubated at 37°C for 5 hours. The resulting fragment of approximately 580bp was purified by Geneclean™ to generate an IL4.Y124D/SmaI/KpnI fragment.

(b) Construction of IgG1 coding region

The COSFcLink vector (Table 1) contains human IgG1 cDNA encoding amino acids 1-4 and 6-15 of the hinge, 1-110 of CH2 and 1-108 of CH3 described by Ellison J., Berson B. and Hood L. E., Nucleic Acids Research vol10, pp4071-4079, 1982. Residue 5 of the hinge is changed from cysteine in the published IgG1 sequence to alanine by alteration of TGT to GCC in the nucleotide sequence. This was cloned from the human IgG plasma cell leukemia ARH-77 (American Type Tissue Collection), using RT-PCR and fully sequenced to confirm identity with the published sequence [patent application publication WO 92/00985]

The construction of COSFc began with a pUC18 vector containing the human IgG1 cDNA above (pUC18-Fc), which was digested with KpnI and SacII, deleting the CH1, hinge and part of CH2. The deleted region was replaced with a PCR

amplified fragment containing the hinge-CH2 region as follows. Using the following PCR primers:

5' TCG AGC TCG GTA CCG AGC CCA AAT CGG CCG ACA AAA CTC ACA
5 C 3'
and
5' GTA CTG CTC CTC CCG CGG CTT TGT CTT G 3'

A DNA fragment containing the hinge-CH2 region was amplified from
10 pUC18-Fc, digested with KpnI and SacII, gel purified and cloned into the KpnI/SacII digested pUC18-Fc vector. The Cys, which occurs at position 230 (Kabat numbering; Kabat et al., "Sequences of Proteins of Immunological Interest, 5th Edition, US Department of Health and Human Services, NIH Publication No. 91-3242 (1991)) of the IgG1 heavy chain, was altered to an Ala through a TGT to GCC substitution in
15 the nucleotide sequence. An altered DNA sequence in one of the PCR primers introduced a unique KpnI site at the 5' end of the hinge. The resulting plasmid was called pUC18Fcmod, and the junctions and PCR amplified region were sequenced for confirmation.

The entire hinge-CH2-CH3 insert in pUC18-Fcmod was removed in a single
20 DNA fragment with KpnI and XbaI, gel purified, and ligated into SFcR1Cos4 cut with KpnI and XbaI to create COSFc.

SFcR1Cos4 is a derivative of pST4DHFR (Deen, K , McDougal, JS, Inacker, R, Folena-Wasserman, G, Arthos, J, Rosenberg, J, Maddon, PJ, Axel, R, and Sweet, RW. Nature 331: 82 [1988]) and contains the soluble Fc receptor type I (sFcR1) inserted between the cytomegalovirus (CMV) promoter and bovine growth hormone (BGH) polyadenylation regions, and also contains the dihydrofolate reductase (DHFR) cDNA inserted between the β-globin promoter and SV40 polyadenylation regions, an SV40 origin of replication, and an ampicillin resistance gene for growth in bacteria. Cutting the vector with KpnI and XbaI removes the sFcR1 coding region, so
25 that the COSFc vector contains the hinge-CH2-CH3 region inserted between the CMV promoter and BGH polyA regions.

The COSFcLink vector was made from COSFc by inserting an oligonucleotide linker at the unique EcoRI site of the vector, which recreates this EcoRI site, and also introduces BstEII, PstI and EcoRV cloning sites. The
30 oligonucleotides used were:

5' AATTGGTACCTGCAGATATCAAGCT 3'
3' GCCAATGGACGTCTATAGTTGATTAA 5'

The junction was sequenced to confirm orientation in the vector. The size of the final vector is 6.37 kb.

5 (c) Construction of DNA coding for fusion protein.

To insert the IL4.Y124D cDNA, the COSFcLink vector was prepared by digesting with EcoRV and KpnI as follows: 5 μ g DNA was incubated with 15 units EcoRV in react 2 at 37°C for 5 hours, followed by ethanol precipitation. The resulting DNA was digested with KpnI in react 4 at 37°C for 3 hours, and ethanol precipitated. The IL4.Y124D/SmaI/KpnI and the COSFcLink/EcoRV/KpnI fragments were ligated together to form plasmid pDB951, which encodes the IL4.Y124D/IgG1 fusion protein. The ligation was achieved using an Amersham DNA ligation kit, product code RPN 1507, the reactions being incubated at 16°C overnight. The ligation reaction products were transformed into Promega JM109 competent cells (high efficiency) and plated onto Luria Broth agar containing ampicillin at 50 μ g/ml. Transformants were cultured in Luria Broth (containing ampicillin at 50 μ g/ml) and DNA prepared using Promega "Magic Minipreps". Production of an IL4.Y124D/COSFcLink recombinant DNA was verified by restriction digests and DNA sequencing. The complete IL4.Y124D sequence and the junctions with the COSFcLink DNA were confirmed by DNA sequencing (Table 2). The coding sequence of the recombinant IL4.Y124D/IgG1 DNA is shown in Table 3 and the amino acid sequence of the fusion protein is shown in Table 4. The IL4.Y124D/COSFcLink recombinant DNA was prepared and purified using caesium chloride gradients and the DNA used to transiently transfect HeLa cells.

25

2. Expression of the fusion protein

HeLa cells were grown in MEM α medium (Gibco) with 10% foetal calf serum and 1% glutamine. For the assay, 1 x 10⁶ HeLa cells were seeded in 15mls RPMI-1640 medium with 10% newborn calf serum, 1% glutamine ("seeding medium"), in a 75cm² flask, four days prior to transfection. On the day prior to transfection, a further 12.5mls seeding medium was added to each flask. On the day of transfection, the medium was changed to 15mls of "transfection medium" (MEM medium with Earle's salts containing 10% newborn calf serum and 1% non essential amino acids), at time zero. At time +3 hours, 25 μ g of the appropriate DNA in 0.125M CaCl₂, 1x HBS (HEPES buffered saline) was added to the cells. At time +7 hours, the cells were subjected to a glycerol shock (15%v/v) and then left to incubate overnight in 12.5mls seeding medium containing 5mM sodium butyrate. The next day the cells were washed with PBS (Dulbecco's phosphate buffered saline) and

12.5mls "harvest medium" (RPMI-1640 with 2% of a 7.5% stock sodium bicarbonate solution) was added. After a further 24 hour incubation, the supernatants were removed, centrifuged at 1000rpm for 5 minutes to remove cell debris and stored at either 4°C or -20°C.

5

3. Biological Activity

For assay of supernatant for IL4 antagonist activity: using the method described in Spits et al., J. Immunology 139, 1142 (1987), human peripheral blood lymphocytes were incubated for three days with phytohaemagglutinin, a T cell mitogen, to upregulate the IL4 receptor. The resultant blast cells were then stimulated for a further three days with IL4. Proliferation was measured by the incorporation of 3H thymidine.

The IL4.Y124D/IgG1 chimera inhibited 3H thymidine incorporation by human peripheral blood T lymphocytes stimulated with 133pM IL4 in a dose dependent manner.

15

Example 2 IL4.Y124D/IgG4 fusion protein

20 1. Construction of DNA coding for fusion protein

PCR was performed to amplify the IL4.Y124D coding region and introduce a silent nucleotide substitution at the 3' end which creates a XhoI site. As substrate for the PCR reaction 20ng of linearised pDB951 plasmid (Example 1.1(c)) was used. The oligonucleotide primers used were as follows:

25

1) 5' CAC AAG TGC GAT ATC ACC TTA CAG GAG ATC 3'
(includes an EcoRV restriction site, GATATC)

30

2) 5' CTC GGT ACC GCT CGA GCA CTT TGA GTC TTT 3'
(includes a XhoI restriction site, CTCGAG).

35

A second PCR reaction was performed to amplify the hinge-CH₂-CH₃ fragment of the human IgG4 heavy chain. The substrate for this was a synthetic human IgG4 heavy chain cDNA, the sequence of which is described in Table 5, and is based on the Genbank sequence GB:HUMIGCD2 (Ellison J., Buxbaum J. and Hood L.E., DNA 1:11-18, 1981). Numerous silent substitutions were made to the published nucleotide sequence. The gene was assembled by combining two 0.5Kb synthetic DNA fragments. Each 0.5Kb fragment was made by annealing a series of

overlapping oligonucleotides and then filling in the gaps by PCR. The two 0.5Kb fragments were joined at the SacII site and inserted into the pCR2 vector. A 1.0Kb ApaI-BglII fragment containing the entire constant region was isolated and ligated into an expression vector, pCD, containing a humanized IL4 specific variable region.

- 5 This construct was used as the PCR substrate to amplify the hinge-CH2-CH3 region of IgG4.

The oligonucleotide primers used for amplification of the IgG4 hinge-CH2-CH3 region were as follows:

10 1) 5' GGT GGA CAA CTC GAG CGA GTC CAA ATA TGG 3'
(includes a XhoI restriction site, CTCGAG)

2) 5' TTA CGT AGA TCT AGA CTA CAC TCA TTT ACC 3'
(includes an XbaI site, TCTAGA).

15 The conditions for both PCR reactions were as described for the derivation of pDB951. Briefly, primers were used at 5ng/μl, and dNTPs at a final concentration of 0.2mM in a total reaction volume of 100μl. 2.5 Units of Taq polymerase enzyme from Advanced Biotechnologies were used and 31 cycles of PCR performed. Cycles consisted of a denaturation step of 1 minute at 94°C, an annealing step of 1 minute 30 seconds at 50°C, and an elongation step of 1 minute 30 seconds at 72°C. On cycle 1 denaturation was extended to 5 minutes and on the final cycle elongation was extended to 7 minutes.

20 PCR products of approximately 700bp (hinge-CH2-CH3 of IgG4) and 400bp (IL4.Y124D) were obtained and purified using the Promega "Magic PCR cleanup" kit. The purified PCR reactions were then digested with the following enzymes to create "sticky ends": XhoI and XbaI for IgG4 and EcoRV and XhoI for IL4.Y124D. The digests were incubated at 37°C for 3 hours and then ethanol precipitated. The resulting DNAs were analysed by gel electrophoresis and gave sizes 30 of approximately 690bp (hinge-CH2-CH3 of IgG4) and 370bp (IL4.Y124D).

25 A vector was prepared into which to ligate the hinge-CH2-CH3 of IgG4 and IL4.Y124D PCR fragments by digesting pDB951 (IL4.Y124D in COSFcLink) with EcoRV and XbaI to remove most of the IL4.Y124D/IgG1 fusion molecule. The only part remaining is approximately 75bp at the 5' end of IL4, which is not present 35 in the IL4.Y124D EcoRV/XhoI fragment produced by PCR amplification. 5μg of pDB951 DNA was digested in a total volume of 30μl using react 2 buffer (GibcoBRL). The resulting 5.8Kb DNA fragment was purified using the Geneclean TM procedure.

The three fragments described (IL4.Y124D EcoRV/XbaI, hinge-CH2-CH3 of IgG4 XbaI/XbaI and the 5.8Kb fragment resulting from EcoRV/XbaI digestion of pDB951) were ligated together to form plasmid pDB952, which encodes the IL4.Y124D/IgG4 fusion protein. The ligation was carried out using a DNA 5 ligation kit from Amersham (product code RPN 1507), incubating the reactions at 16° C overnight. The ligation reaction products were transformed into Promega JM109 competent cells (high efficiency) and plated onto Luria Broth agar containing ampicillin at 50µg/ml. Transformants were cultured in Luria Broth (containing ampicillin at 50µg/ml) and DNA prepared using Promega "Magic Minipreps".
10 Production of an IL4.Y124D/IgG4 recombinant DNA was verified by restriction digests, and the complete IL4.Y124D and hinge-CH2-CH3 IgG4 regions were verified by DNA sequencing. Table 6 describes the sequence of the coding region only of the IL4.Y124D/IgG4 fusion molecule, and Table 7 contains the amino acid sequence of the fusion protein. The IL4.Y124D/IgG4 recombinant DNA was
15 prepared and purified using caesium chloride gradients and the DNA used to transiently transfect HeLa cells.

2. Expression of the fusion protein

HeLa cells were grown in MEMα medium (Gibco) with 10% foetal calf serum and 1% glutamine. For the assay, 1 x 10⁶ HeLa cells were seeded in 15mls RPMI-1640 medium with 10% newborn calf serum, 1% glutamine ("seeding medium"), in a 75cm² flask, four days prior to transfection. On the day prior to transfection, a further 12.5mls seeding medium was added to each flask. On the day of transfection, the medium was changed to 15mls of "transfection medium" (MEM medium with Earle's salts containing 10% newborn calf serum and 1% non essential amino acids), at time zero. At time +3 hours, 25µg of the appropriate DNA in 0.125M CaCl₂, 1x HBS (HEPES buffered saline) was added to the cells. At time +7 hours, the cells were subjected to a glycerol shock (15%v/v) and then left to incubate overnight in 12.5mls seeding medium containing 5mM sodium butyrate. The next 30 day the cells were washed with PBS (Dulbecco's phosphate buffered saline) and 12.5mls "harvest medium" (RPMI-1640 with 2% of a 7.5% stock sodium bicarbonate solution) was added. After a further 24 hour incubation, the supernatants were removed, centrifuged at 1000rpm for 5 minutes to remove cell debris and stored at either 4°C or -20°C.

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3. Biological Activity

For assay of supernatant for IL4 antagonist activity: using the method described in Spits et al., J. Immunology 139, 1142 (1987), human peripheral blood lymphocytes were incubated for three days with phytohaemagglutinin, a T cell

mitogen, to upregulate the IL4 receptor. The resultant blast cells were then stimulated for a further three days with IL4. Proliferation was measured by the incorporation of 3H thymidine.

5 The IL4.Y124D/IgG4 chimera inhibited 3H thymidine incorporation by human peripheral blood T lymphocytes stimulated with 133pM IL4 in a dose dependent manner.

Example 3

IL4.Y124D/IgG4 PE fusion protein

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1. Construction of DNA coding for fusion protein

PCR is performed to amplify the IL4.Y124D coding region and introduce a silent nucleotide substitution at the 3' end which creates a XhoI site as described in Example 2.

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A second PCR reaction is performed to amplify the hinge-CH2-CH3 fragment of the human IgG4 heavy chain PE variant. In IgG4 PE, residue 10 of the hinge (residue 241, Kabat numbering) is altered from serine (S) in the wild type to proline (P) and residue 5 of CH2 (residue 248, Kabat numbering) is altered from leucine (L) in the wild type to glutamate (E). Angal S., King D.J., Bodmer M.W.,

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Turner A., Lawson A.D.G., Roberts G., Pedley B. and Adair R., Molecular Immunology vol30pp105-108, 1993, describe an IgG4 molecule where residue 241 (Kabat numbering) is altered from serine to proline. This change increases the serum half-life of the IgG4 molecule.

25

The IgG4 PE variant was created using PCR mutagenesis on the synthetic human IgG4 heavy chain cDNA described in Table 5, and was then ligated into the pCD expression vector. It was this plasmid which was used as the substrate for the PCR reaction amplifying the hinge-CH2-CH3 fragment of IgG4 PE. The sequence of the IgG4 PE variant is described in Table 8. The residues of the IgG4 nucleotide sequence which were altered to make the PE variant are as follows:

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referring to Table 8:

residue 322 has been altered to "C" in the PE variant from "T" in the wild type;

residue 333 has been altered to "G" in the PE variant from "A" in the wild type; and

35

residues 343-344 have been altered to "GA" in the PE variant from "CT" in the wild type.

Oligonucleotide primers are used for amplification of the IgG4 PE variant hinge-CH2-CH3 region as described for the derivation of pDB952.

PCR products of approximately 700bp (hinge-CH2-CH3 of IgG4 PE mutant) and 400bp (IL4.Y124D) are obtained and purified using the Promega "Magic PCR cleanup" kit. The purified PCR reactions are then digested with the following enzymes to create "sticky ends": Xhol and XbaI for IgG4 PE and EcoRV and Xhol for IL4.Y124D. The digests are incubated at 37°C for 3 hours and then ethanol precipitated. The resulting DNAs are of sizes of approximately 690bp (hinge-CH2-CH3 of IgG4 PE) and 370bp (IL4.Y124D).

To obtain larger amounts of the IgG4 PE variant hinge-CH2-CH3 fragment and the IL4.Y124D fragment, the purified and digested PCR products are ligated into Bluescript KS⁺TM which is prepared by digestion with either XhoI and XbaI for the hinge-CH2-CH3 of IgG4 PE fragment or EcoRV and XhoI for the IL4.Y124D fragment, followed by GenecleanTM. A Bluescript KS⁺/hinge-CH2-CH3 of IgG4 PE recombinant and a Bluescript KS⁺/IL4.Y124D recombinant are thus generated. Large amounts of these DNAs are produced using the Promega "Magic Maxiprep" method. The IgG4 PE hinge-CH2-CH3 fragment is excised from the Bluescript recombinant using XhoI and XbaI. The resulting fragment of approximately 690bp is purified by GenecleanTM to generate large amounts of the IgG4 PE hinge-CH2-CH3 XhoI/XbaI fragment. The IL4.Y124D fragment is excised from the Bluescript recombinant using EcoRV and XhoI and the resulting fragment of approximately 370bp is purified by GenecleanTM.

A vector is prepared into which to ligate the hinge-CH2-CH3 of IgG4 PE and IL4.Y124D fragments by digesting pDB951 with EcoRV and XbaI as described for the derivation of pDB952.

The three fragments described (IL4.Y124D EcoRV/XhoI, hinge-CH2-CH3 of IgG4 PE variant XhoI/XbaI and the 5.8Kb fragment resulting from EcoRV/XbaI digestion of pDB951) are ligated together to form plasmid pDB953 using a DNA ligation kit from Amersham (product code RPN 1507), incubating the reactions at 16°C overnight. The ligation reaction products are transformed into Promega JM109 competent cells (high efficiency) and plated onto Luria Broth agar containing ampicillin at 50µg/ml. Transformants are cultured in Luria Broth (containing ampicillin at 50µg/ml) and DNA prepared using Promega "Magic Minipreps". Production of an IL4.Y124D/IgG4 PE variant recombinant DNA is verified by restriction digests, and the complete IL4.Y124D and hinge-CH2-CH3 IgG4 PE variant regions are verified by DNA sequencing. Table 9 describes the sequence of the coding region only of the IL4.Y124D/IgG4 PE fusion molecule, and Table 10 contains the amino acid sequence of the fusion protein. The IL4.Y124D/IgG4 PE recombinant DNA is prepared and purified using caesium chloride gradients and the DNA used to transiently transfect HeLa cells.

2. Expression of the fusion protein

5 HeLa cells were grown in MEM α medium (Gibco) with 10% foetal calf serum and 1% glutamine. For the assay, 1×10^6 HeLa cells were seeded in 15mls RPMI-1640 medium with 10% newborn calf serum, 1% glutamine ("seeding medium"), in a 75cm² flask, four days prior to transfection. On the day prior to transfection, a further 12.5mls seeding medium was added to each flask. On the day 10 of transfection, the medium was changed to 15mls of "transfection medium" (MEM medium with Earle's salts containing 10% newborn calf serum and 1% non essential amino acids), at time zero. At time +3 hours, 25 μ g of the appropriate DNA in 0.125M CaCl₂, 1x HBS (HEPES buffered saline) was added to the cells. At time +7 hours, the cells were subjected to a glycerol shock (15%v/v) and then left to incubate 15 overnight in 12.5mls seeding medium containing 5mM sodium butyrate. The next day the cells were washed with PBS (Dulbecco's phosphate buffered saline) and 12.5mls "harvest medium" (RPMI-1640 with 2% of a 7.5% stock sodium bicarbonate solution) was added. After a further 24 hour incubation, the supernatants were removed, centrifuged at 1000rpm for 5 minutes to remove cell debris and stored at 20 either 4°C or -20°C.

3. Biological Activity

For assay of supernatant for IL4 antagonist activity: using the method described in Spits et al., J. Immunology 139, 1142 (1987), human peripheral blood lymphocytes were incubated for three days with phytohaemagglutinin, a T cell mitogen, to upregulate the IL4 receptor. The resultant blast cells were then stimulated for a further three days with IL4. Proliferation was measured by the incorporation of 3H thymidine.

The IL4.Y124D/IgG4 PE chimera inhibited 3H thymidine incorporation by 30 human peripheral blood T lymphocytes stimulated with 133pM IL4 in a dose dependent manner.

Example 4. Mammalian Expression vector containing DNA coding for IL4.Y124D/IgG4 PE.

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1. Construction of DNA

The pCDN vector (Aiyar, N., Baker, E., Wu, H-L., Nambi, P., Edwards, R.M., Trill, J.J., Ellis, C., Bergsma, D. Molecular and Cellular Biochemistry 131:75-86, 1994) contains the CMV promoter, a polylinker cloning region, and the BGH polyadenylation

region. This vector also contains a bacterial neomycin phosphotransferase gene (NEO) inserted between the β -globin promoter and SV40 polyadenylation region for GeneticinTM selection, the DHFR selection cassette inserted between the β -globin promoter and BGH polyadenylation region for methotrexate (MTX) amplification, an ampicillin resistance gene for growth in bacteria, and a SV40 origin of replication.

To insert the IL4.Y124D/IgG4 PE cDNA, the pCDN vector was prepared by digesting with NdeI and BstX1 as follows: 15 μ g of DNA was incubated with 30 units of BstX1 in react 2 (Gibco-BRL) at 55°C for 1 hour, and ethanol precipitated. The resulting DNA was digested with NdeI in react 2 at 37°C for 1 hour, and ethanol precipitated. The IL4.Y124D/IgG4 PE fragment was prepared from pDB953 (Example 3.1) by digesting with BstX1 and NdeI as follows: 15 μ g of DNA was incubated with 30 units of BstX1 in react 2 at 55°C for 1 hour, and ethanol precipitated. The resulting DNA was digested with NdeI in react 2 at 37°C for 1 hour, and ethanol precipitated.

The IL4.Y124D/IgG4 PE NdeI/BstX1 and pCDN NdeI/BstX1 fragments were ligated together to form the plasmid pCDN-IL4.Y124D/IgG4 PE. The ligation was achieved using 2 units of T4 DNA Ligase (Gibco BRL) with T4 DNA Ligase buffer. The reactions were incubated at 16°C overnight. The ligation reaction products were transformed into Gibco-BRL DH5a competent cells (subcloning efficiency) and plated onto Luria Broth agar containing 75 ug/ml ampicillin. Transformants were cultured in Luria Broth (containing ampicillin at 50 ug/ml) and DNA prepared by alkaline lysis. Production of a pCDN-IL4.Y124D/IgG4 PE DNA was confirmed by restriction digests. The complete sequence of the recombinant IL4.Y124D/IgG4 PE DNA was confirmed by sequencing. The pCDN-IL4.Y124D/IgG4 PE recombinant DNA was prepared and purified using Qiagen columns and the DNA was used to transiently infect COS cells and electroporated into CHO cells to create stable clones.

2. Expression of the Fusion Protein

a) Transient Expression in COS

COS-1 cells were grown in DMEM medium with 10% fetal bovine serum. For the transfection, cells were seeded at 2 X 10⁵ cells into a 35mm tissue culture dish 24 hours prior. A solution containing 1 μ g of DNA in 100 μ l of DMEM without serum is added to a solution containing 6 μ l of LIPOFECTAMINE Reagent (Gibco-BRL) in 100 μ l of DMEM without serum, gently swirled and incubated at room temperature for 45 minutes. The cells are washed once with serum free DMEM. 0.8ml of serum free DMEM is added to the DNA-LIPOFECTAMINE SOLUTION, mixed gently and the diluted solution is overlayed on the cells. The cells are incubated at 37°C for 5 hours, then 1ml of DMEM containing 20% fetal bovine serum is added. The cells are assayed 48-72 hours later to determine expression levels.

b) **Electroporation into CHO cells**

CHO cells, ACC-098 (a suspension cell line derived from CHO DG-44, Urlaub, G.,
5 Kas, E., Carothers, A.M. and Chasin, L.A. Cell, 33. 405-412, 1983) were grown in
serum free growth medium WO 92/05246. 15 μ g of the pCDN-IL4.Y124D/IgG4 PE
plasmid was digested using 30 units of NotI at 37°C for 3 hours to linearize the
10 plasmid, and precipitated with ethanol. The resulting DNA was resuspended in 50 μ l
of 1 X TE (10mM Tris, pH 8.0, 1mM EDTA). The DNA was electroporated into 1 X
10⁷ ACC-098 cells, using a Bio Rad Gene Pulser set at 380V and 25 μ Fd. The cells
were resuspended into growth medium at 2.5 X 10⁴ cells/ml, and 200 μ l of the cell
suspension was plated into each well of a 96 well plate. 48 hours later the medium
was switched to growth medium containing 400 μ g/ml G418 (Geneticin). Twenty one
15 days post selection, conditioned medium from the colonies which arose were screened
by Elisa assay. The highest expressing colonies were transferred to 24 well plates in
order to be scaled up.

Table 1. DNA sequence of COSFcLink vector, 6367bp

SEQ ID No:1	
GACGTCGACGGATCGGGAGATCGGGGATCGATCCGTCGACGTACGACTAGTTATTAAATAG	60
5 TAATCAATTACGGGGTCAATTAGTCATAGCCCATAATATGGAGTTCGCCTACATAACTT	120
ACGGTAAATGGCCCGCTGGCTGACGCCAACGACCCCCGCCATTGACGTCAATAATG	180
ACGTATGTTCCCATACTAGAACGCAATAGGGACTTCCATTGACGTCAATGGGTGGACTAT	240
TTACGGTAAACTGCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCT	300
10 ATTGACGTCAATGACGGTAATGGCCCGCTGGCATTATGCCAGTACATGACCTTATGG	360
GACTTCCACTTGGCAGTACATCACGTATTAGTCATCGCTATTACCATGGTATGCGG	420
TTTGGCAGTACATCAATGGCGTGGATAGCGGTTTGACTCACGGGATTCCAAGTCTC	480
CACCCCATTGACGTCAATGGAGTTGGCACC AAAATCAACGGGACTTCCAAA	540
TGTCGTAACAACCTCCGCCCATGACGAAATGGCGGTAGGCGTGTACGGTGGAGGTC	600
TATATAAGCAGAGCTGGGTACGTGAACCGTACATGCCCTGGAGACGCCATGAATTGG	660
15 TTACCTGCAAGATACTAAGCTAATTGGTACCGAGCCAAATCGGCCGACAAAACAC	720
ATGCCACCGTGGCCAGCACCTGAACCTCTGGGGGACCGTCAGTCCTCTTCCCCC	780
AAAACCAAGGACACCCCTCATGATCTCCGGACCCCTGAGGTACATGCGTGGTGGGAGA	840
CGTGAGCCACGAAGACCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCA	900
TAATGCCAAGACAAAGCCGGAGGAGCAGTACAACAGCACGTACCGGTGGTACCGT	960
20 CCTCACCGTCCTGCACCAAGGACTGGCTGAATGGCAAGGAGTACAAGTCAAGGTCTCAA	1020
CAAAGCCCTCCCAGCCCCATCGAGAAAACATCTCCAAAGCCAAGGGCAGCCCCGAGA	1080
ACCACAGGTGTACACCCCTGCCCATCCGGATGAGCTGACCAAGAACAGGTCAGCCT	1140
GACCTGCCCTGGTCAAAGGCTTCTATCCCAAGGCCATGCCGTGGAGTGGAGAGCAATGG	1200
GCAGCCGGAGAACAACTACAAGACCAAGCAGCCTCCGTGCTGGACTCCGACGGCTCTTCTT	1260
25 CCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTCTCATG	1320
CTCCGTATGCATGAGGCTCTGCACAACCAACTACACGCCAGAACAGCCTCTCCGTCTCC	1380
GGGTAAATGAGTGTAGCTAGAGCTCGCTGATCAGCCTCGACTGTGCCCTCTAGTTGCCA	1440
GCCATCTGTTGTTGCCCTCCCCGTGCTCCTTGACCCCTGGAAGGTGCCACTCCCAC	1500
TGTCCTTCTAATAAAATGAGGAAATTGATCGCATTGTCAGTGTAGGTGTCAATTCTAT	1560
30 TCTGGGGGTGGGTGGGGCAGGACAGCAAGGGGAGGATTGGGAAGACAATAGCAGGCA	1620
TGCTGGGGATGCGGTGGCTCTATGGAACCAAGCTGGGCTCGAGGGGGATCTCCGATC	1680
CCCAGCTTGCTCTCAATTCTATTGCTAAATGAGAAAAAAAGGAAAATTAAATTAA	1740
ACACCAATTCACTAGTTGATTGAGCAAATGCGTGGCAAAAGGATGTTAGAGACAGT	1800
GTTCTCTGCACAGATAAGGACAACATTATTCAAGAGGGAGTACCCAGAGCTGAGACTCCT	1860
35 AAGCCAGTGAAGTGGCACAGCATTCTAGGGAGAAATATGCTGTACACCGAAGCCTGAT	1920
TCCGTAGAGCCACACCTTGGTAAGGGCAATCTGCTCACACAGGATAGAGAGGGCAGGAG	1980
CCAGGGCAGAGCATATAAGGTAGGATCAGTTGCTCCTCACATTGCTTCTGACAT	2040
AGTTGTGTGGAGCTGGATAGCTGGACAGCTCAGGGCTGCGATTGCGCCAAACTT	2100
GACGGCAATTCTAGGTGAAGGCTGGTAGGATTATCCCCGTGCCATCATGGTTCGAC	2160
40 CATTGAACTGCATCGCGTGTCCAAAATATGGGATTGGCAAGAACGGAGACCTAC	2220
CCTGGCCTCCGTCAGGAACGAGTTCAAGTACTTCAAGAACATGCTCCATTCTGAGA	2280
TGGAAGGTAACAGAAATCTGGTATTATGGTAGGAAACCTGGTCTCCATTCTGAGA	2340
AGAATCGACCTTAAAGGACAGAATTAAATAGTTCTCAGTAGAGAACTCAAAGAACAC	2400
CACGAGGAGCTATTCTGGCAAAAGTTGGATGATGCCATTAGACTATTGAACAAC	2460
45 CGGAATTGGCAAGTAAAGTAGACATGGTTGGATAGTCGGAGGAGTTCTGTTACCAAGG	2520
AAGCCATGAATCAACCAAGGCCACCTTAGACTCTTGTGACAAGGATCATGCAGGAATTG	2580
AAAGTGACACGTTTCCCAGAAATTGATTGGGGAAATATAAACTCTCCAGAACATACC	2640
CAGGCGTCTCTCTGAGGTCCAGGAGGAAAAGGCATCAAGTATAAGTTGAAGTCTACG	2700
AGAAGAAAAGACTAACAGGAAGATGTTCAAGTCTGCTCCCTCAAAGCTATGCA	2760
50 TTTTATAAGACCATGCTAGCTGAACCTGGTTATTGAGCTTATAATGGTTACAATAA	2820
AGCAATAGCATCACAAATTTCACAAATAAGCATTGTTCTACTGCTGGATCAACGATAGCTTATGTTGGG	2880
TTGTCCAACATCATCAATGTATCTTATCATGCTGGATCAACGATAGCTTATGTTGGG	2940
GATGCCAAGCACCTGGATGCTGGTTCTGCTACTGATTAGGCCATTGCC	3000

	TGAGTGGGGCTGGGAGCACTAACTTCTTTCAAAGGAAGCAATGCAGAAAGAAAAGC	3060
	ATACAAAGTATAAGCTGCATGTAAATAATGAAAGAAGATAAGGTTGTATGAATTAGATT	3120
	ACATACTTCTGAATTGAAACTAAACACCTTAATTCTAAATATAACACATTTCATA	3180
	TGAAAGTATTTACATAAGTAACTCAGATACATAGAAAACAAAGCTAATGATAGGTGTCC	3240
5	CTAAAAGTTCATTTATTACAAATGATGAGCTGGCCATCAAATTCCAGCTCAAT	3300
	TCTCAACGAATTAGAAAGAGCAACTGCAAACCTCATCTGAAATAACAAAAACCTAGGA	3360
	TAGCAAAAACCTCTCAAGGATAAAAGAACCTCTGGTGGAAATCACCATGCCTGACCTAA	3420
	AGCTGTACTACAGAGCAATTGTGATAAAAACCTGCATGGTACTGATATAGAAACGGACAAG	3480
	TAGACCAATGGAATAGAACCCACACACCTATGGTCACTTGATCTCAACAAGAGAGCTAA	3540
10	AACCATCCACTGGAAAAAGACAGCATTTCACAAATGGTGTGGCACAACGGTGGTT	3600
	ATCATGGAGAAGAATGTGATTGATCCATTCCAATCTCTTGTACTAAGGTCAAATCTAA	3660
	GTGGATCAAGGAACCTCCACATAAAACAGAGACACTGAAACTTATAGAGGAGAAAGTGGG	3720
	GAAAAGCCTCGAAGATATGGGACAGGGGAAAATTCTGAATAGAACAGCAATGGCTTG	3780
15	TGCTGTAAAGATCGAGAATTGACAAATGGGACCTCATGAAACTCCAAAGCTATCGGATCAA	3840
	TTCCTCCAAAAAGCCTCCTCACTACTTCTGGAAATAGCTCAGAGGCCAGGCCCTCGG	3900
	CCTCTGCATAAAATAAAAAAAATTAGTCAGCCATGCATGGGCGGAGAATGGGCGGAACTG	3960
	GGCGGAGTTAGGGCGGGATGGCGGAGTTAGGGCGGGACTATGGTGTGACTAATTG	4020
	AGATGCATGCTTGCATACTCTGCTGCTGGGAGCCTGGGACTTCCACACCTGGTT	4080
20	GCTGACTAATTGAGATGCATGCTTGACACTTCTGCCTGCTGGGAGCCTGGGACTTT	4140
	CCACACCCCTAATGACACACATTCCACAGAATTAACTCCGATCCCGTCGACCTCGAGAG	4200
	CTTGGCGTAATCATGGTCATAGCTGTTCCCTGTGAAATTGTTATCCGTCACAAATTCC	4260
	ACACAACATACGAGCCGGAAAGCATAAAAGTGTAAAGCCTGGGTGCCTAATGAGTGAGCTA	4320
	ACTCACATTAATTGCGTTGCGTCACTGCCCGCTTCCAGTCGGAAACCTGTCGTGCCA	4380
	GCTGCATTAATGAATCGGCCAACCGCGGGGGAGAGGCGGTTGCGTATTGGCGCTCTTC	4440
25	CGCTTCCTCGCTCACTGACTCGCTCGCTCGGTGCGCTCGGGCTGCGGAGCGGTATCAGC	4500
	TCACTCAAAGCGGTAAATACGGTTATCCACAGAAATCAGGGATAACGCAAGGAAAGAACAT	4560
	GTGAGCAAAGGCCAGCAAAGGCCAGGAACCGTAAAAGGCCCGTGTGCTGGCGTTTT	4620
	CCATAGGCTCGCCCCCTGACGAGCATCACAAAAATCAGCCTCAAGTCAGAGGTGGCG	4680
	AAACCCGACAGGACTATAAAGATACCAGCGTTCCCCCTGGAAGCTCCCTGTCGCGCTC	4740
30	TCCCTGTTCCGACCCCTGCCGTTACCGGATACCTGTCGCCCTTCTCCCTCGGAAAGCGT	4800
	GGCGCTTCTCAATGTCACGCTGTAGGTATCTCAGTTGGTGTAGTCGTTGCTCCAA	4860
	GCTGGGCTGTGTCACGAACCCCCCGTTACGCCGACCGCTGCCCTATCCGTAACTA	4920
	TCGTCTTGAGTCCAACCCGTAAGACACGACTTATGCCACTGGCAGCAGCCACTGGTAA	4980
	CAGGATTAGCAGAGCAGGTTATGAGGCGGTGCTACAGAGTTCTGAAGTGGTGGCTAA	5040
35	CTACGGCTACACTAGAAGGACAGTATTGGTATCTGCCTGCTGAAGCCAGTTACCTT	5100
	CGGAAAAAGAGTTGGTAGCTCTTGATCCGCAACAAACACCAGCTGGTAGCGGTGGTT	5160
	TTTGTTGCAAGCAGCAGATTACCGCAGAAAAAAAGGATCTCAAGAAGATCCTTGAT	5220
	CTTTCTACGGGCTGACGCTCAGTGGAACGAAAACCTCACGTTAAGGATTGGTCA	5280
	GAGATTATCAAAGGATCTTACCTAGATCTTTAAATTAAAAAGTAAAGTTAAATC	5340
40	AATCTAAAGTATATGAGTAAACTGGTCTGACAGTACCAATGCTTAATCAGTGAGGC	5400
	ACCTATCTCAGCGATCTGCTATTGTTCATCCATAGTGCCTGACTCCCCGTCGTGTA	5460
	GATAACTACGATAACGGGAGGGCTACCATCTGCCCGAGTGCTGAATGATACCGCGAGA	5520
	CCCACGCTACCGGCTCAGATTTACGCAATAACCAAGCCAGCCAGCGGAAGGGCCGAGCG	5580
	CAGAAGTGGCTGCAACTTATGCCCTCCATCCAGTCATTAAATTGTCGCCGGAAAGC	5640
45	TAGAGTAAGTAGTCGCCAGTTAATAGTTGCGAACGTTGTTGCCATTGCTACAGGCAT	5700
	CGTGGTGTACGCTCGTGTGGTATGGCTTCATTAGCTCAGCTCCGGTCTCCACGATCAAG	5760
	GCGAGTTACATGATCCCCCATGTTGCAAAAAGCGGTAGCTCTCGGTCTCCGAT	5820
	CGTTGTCAGAAGTAAGTGGCGCAGTGTATCACTCATGGTATGGCAGCAGTCATAA	5880
	TTCTCTTACTGTCATGCCATCGTAAGATGTTCTGACTGGTGAAGTACTCAACCAA	5940
50	GTCATTCTGAGAATAGTGTATGCGGCACCGAGGTGCTCTGGCCGGCTCAATACGGGA	6000
	TAATACCGGCCACATAGCAGAACCTTAAAGGCTCATATTGAAAAGCTTCTCGGG	6060
	GCGAAAAACTCTCAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGC	6120
	ACCCAACTGATCTCAGCATTTTACTTCACCGCTTCTGGGTGAGCAGGAAAACAGG	6180
	AAGGCAAAATGCCGAAAAAGGAATAAGGCGACACGGAAATGTTGAATACTCATCT	6240

CTTCCTTTCAATATTATTGAAGCATTATCAGGGTTATTGCTCATGAGCGGATAACAT	6300
ATTTGAATGTATTTAGAAAAATAACAAATAGGGTCCGCGCACATTCGGGAAAAGT	6360
GCCACCT	6367

5

Table 2. DNA sequence of encoded Y124D-IgG1 fusion molecule in COSFcLink vector, 6926bp

SEQ ID No:2		
10	GACGTCGACGGATGGGAGATCGGGATCGATCCGTCGACGTACGACTAGTTATTAATAG	60
	TAATCAATTACGGGGTCATTAGTTCATAGCCCATATATGGAGTCCCGTTACATAACTT	120
	ACGGTAAATGGCCCGCTGGCTGACCGCCCAACGACCCCCGCCATTGACGTCAATAATG	180
	ACGTATGTTCCCATACTAGTAACCCAATAGGGACTTCCATTGACGTCAATGGGTGGACTAT	240
	TTACGGTAAACTGCCCACCTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCT	300
15	ATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCAGTACATGACCTTATGG	360
	GACTTTCTACTTGGCAGTACATCTACGTATTAGTCATCGTATTACCATGGTGTGCGG	420
	TTTGGCAGTACATCAATGGCGTGGATAGCGGTTGACTCACGGGATTCCAAGTCTC	480
	CACCCCATGGACGTCAATGGGAGTTGTTGGCACAAAATCAACGGGACTTCCAAAAA	540
	TGTCGAACAACCTCCGCCCCATTGACGCAAATGGCGGTAGGCGTACGGTGGGAGGTC	600
20	TATATAAGCAGAGCTGGGTACGTGAACCGTCAGATGCCCTGGAGACGCCATGAATTGG	660
	TTACCTGCAAGATGGGCTCCAGGAATTCCGCATTGCAAGAGATAATTGTATTAAGTGCCTA	720
	GCTCGATACAATAAACGCCATTGACCATTACACATGGTGTGACCTCCAAGCTTAC	780
	CTGCCATGGGTCTCACCTCCAACTGCTTCCCCCTCTGTTCTCCTGCTAGCATGTGCCG	840
	GCAACTTTGTCCACGGACACAAGTGCATATCACCTACAGGAGATCATAAAACTTGA	900
25	ACAGCCTCACAGAGCAGAAGACTCTGTCACCGAGTTGACCGTAACAGACATCTTGCTG	960
	CCTCCAAGAACACAACACTGAGAAGGAAACCTCTGCAGGGCTGCGACTGTGCTCCGGCAGT	1020
	TCTACAGCACCAGTGAAGGACACTCGCTGCCCTGGTGCAGTGCACAGCAGTTCCACA	1080
	GGCACAAGCAGCTGATCCGATTCTGAAACGGCTCGACAGGAACCTCTGGGGCTGGCGG	1140
	GCTTGAATTCTGTCTGTGAAGGAAGCAACCAAGAGTACGTTGGAAAACCTCTGGAAA	1200
30	GGCTAAAGACGATCATGAGAGAGAAAGACTCAAAGTGTGAGCGGTACCGAGCCAAAT	1260
	CGGCCGACAAAACCTCACACATGCCACCGTGCCCAGCACCTGAACCTCTGGGGGACCGT	1320
	CAGTCTTCTCTTCCCCCCTAAACCCAAGGACACCCCTCATGATCTCCGGACCCCTGAGG	1380
	TCACATGCGTGGTGGGACGTGAGCCACGAAGACCCCTGAGGTCAAGTCAACTGGTACG	1440
	TGGACGGCGTGGAGGTGCATAATGCAAGACAAAGCCGGGAGGAGCAGTACAACAGCA	1500
35	CGTACCGGGTGGTCAGCGTCTCACCGTCTGCACCAGGACTGGCTGAATGGCAAGGAGT	1560
	ACAAGTGCAGGTCTCAACAAAGCCCTCCAGCCCCCATCGAGAAAACCATCTCAAAG	1620
	CCAAAGGGCAGCCCCGAGAACACAGGTGACACCCCTGCCCTATCCGGATGAGCTGA	1680
	CCAAGAACACAGGTGACGCTGACCTGCTGGTCAAAGGCTCTATCCAGCGACATGCCG	1740
	TGGAGTGGGAGAGCAATGGCAGCCGGAGACAACACTACAAGACCAACGCCCTCCGTGCTGG	1800
40	ACTCCGACGGCTCTTCTTACAGCAAGCTCACCGTGGACAAGAGCAGGGTGGCAGC	1860
	AGGGGAACGTCTCTCATGCTCCGTGATGCATGAGGCTCTGCACAAACCACTACACGCAGA	1920
	AGAGCCTCTCCGTCTCCGGTAAATGAGTGTAGTCTAGAGCTGCTGATCAGCCTCGA	1980
	CTGTCCTCTAGTTGCCAGCCATCTGTTGCTTGCCTCCCTCCCGTGCCTTCTGACCC	2040
	TGGAAGGTGCCACTCCACTGTCCTTCTAATAAAATGAGGAATTGCATCGCATTGTC	2100
45	TGAGTAGGTGTCATTCTATTCTGGGGGTGGGTGGGCAGGACAGCAAGGGGGAGGATT	2160
	GGGAAGACAATAGCAGGCATGCTGGGATGCGGTGGGTCTATGAAACCAGCTGGGCTC	2220
	GAGGGGGGATCTCCGATCCCCAGCTTGCTTCTCAATTCTTATTGATAATGAGAAA	2280
	AAAAGGAAAATTAAATTAAACACCAATTCAAGTACGTTGATTGAGCAAATGCGTGCCTAA	2340
	AGGATGCTTAGAGACAGTGTCTCTGACAGATAAGGACAAACATTATTCAAGGGAGT	2400
50	ACCCAGAGCTGAGACTCTAAGCCAGTGAGTGGCACAGCATTCTAGGGAGAAATATGCTT	2460
	GTCACTACCGAAGCCTGATTCCGTAGAGCCACACCTGGTAAGGGCAATGCTCACAC	2520

	AGGATAGAGAGGGCAGGGCAGGGCAGAGCATATAAGGTGAGGTAGGATCAGTTGCTCC	2580
	TCACATTGCTCTGACATAGTGTGTTGGGAGCTGGATAGCTGGACAGCTCAGGGCT	2640
	GCGATTCGCGCCAAACTTGACGGCAATCCTAGCGTAAGGCTGGTAGGATTTATCCCC	2700
	GCTGCCATCATGGTCGACCATTGAACTGCATCGTCGCCGTGTCCTAAATATGGGATT	2760
5	GGCAAGAACGGAGACCTACCCCTGGCTCGCAGGAACGAGTTCAAGTACTTCAAAGA	2820
	ATGACCACAACCTCTTCAGTGGAAAGTAAACAGAACTCGGTGATTATGGTAGGAAAACC	2880
	TGGTTCTCATTCTGAGAAGAATCGACCTTAAAGGACAGAATTAATATAGTCTCAGT	2940
	AGAGAACTCAAAGAACCAACGAGGAGCTATTTCTGCCAAAGTTGGATGATGCC	3000
	TTAAGACTTATTGAACAACCGGAATTGGCAAGTAAAGTAGACATGGTTGGATAGTCGGA	3060
10	GGCAGTTCTGTTACCAGGAAGCCATGAATCAACCAGGCCACCTAGACTCTTGACAA	3120
	AGGATCATGCAGGAATTGAAAGTGCACGTTTCCCAGAAATTGATTGGGAAATAT	3180
	AAACTCTCCCAGAATACCCAGGCCTCTCTGAGGTCCAGGAGGAAAAGGCATCAAG	3240
	TATAAGTTGAAGTCTACAGAGAAAGACTAACAGGAAGATGCTTCAGTTCTGCT	3300
	CCCTCCTAAAGCTATGCAATTATAAGACATGCTAGCTGAACCTGTTATTGCAGC	3360
15	TTATAATGGTACAAATAAGCAATAGCATCACAAATTTCACAATAAAGCATTTC	3420
	ACTGCATTCTAGTTGTGGTTGTCCAAACTCATCAATGTATCTTATCATGCTGGATCAA	3480
	CGATAGCTTATCTGTGGCGATGCCAACCTGGATGCTTGGTTCTGCTACTGAT	3540
	TTAGAAGCATTGCCCTGAGTGGGCTTGGGAGCACTAACTTCTCTTCAAAGGAA	3600
	GCAATGCAGAAAGAAAAGCATACAAAGTATAAGTGCCTAGTAATAATGGAAGAAGATAA	3660
20	GGTGTATGAATTAGATTACATACACTTCTGAATTGAAACTAACACCTTAAATTCTAA	3720
	ATATATAACACATTTCATATGAAAGTATTTCACATAAGTAACCTCAGATACATAGAAAACA	3780
	AAGCTAATGATAGGTGTCCCTAAAGTTCAATTATAATTCTACAAATGATGAGCTGGCC	3840
	ATCAAAATTCCAGCTCAATTCTCAACGAAATTAGAAAGAGAAATCTGCAAACCTCATCTGG	3900
	AATAACAAAAACCTAGGATAGCAAAACACTCTCTCAAGGATAAAAGAACCTCTGGTGGAA	3960
25	ATCACCATGCCTGACCTAAAGCTGACTACAGAGCAATTGTGATAAAACTGCATGGTAC	4020
	TGATATAGAAACGGACAAGTAGACCAATGGAATAGAACCCACACACCTATGGTCACTTGA	4080
	TCTTCAACAAGAGAGCTAAACCATCCACTGGAAAAAGACAGCATTCAACAAATGGT	4140
	GCTGGCACAACTGGTGGTATCATGGAGAAGAATGTGAATTGATCCATTCAAATCTCCTT	4200
	GTACTAAGGTCAAATCTAAGTGGATCAAGGAACCTCCACATAAAACCAAGAGACACTGAAAC	4260
30	TTATAGAGGAGAAAGTGGGAAAGCCTCGAAGATATGGGACAGGGAAAAATTCTGA	4320
	ATAGAACAGCAATGGCTTGTGCTGAAGATCGAGAATTGACAATGGACCTCATGAAAC	4380
	TCCAAAGCTATCGGATCAATTCTCCTCCTCCTACTACTCTGGAATAGCTCA	4440
	GAGGCCGAGGGCGCTGGCCTCTGCTGCTAAATAAAAAAAATTAGTCAGCCATGCATGGG	4500
	CGGAGAAATGGCGGAACTGGCGGAGTTAGGGCGGGATGGCGGAGTTAGGGCGGGAC	4560
35	TATGGTTGCTGACTAATTGAGATGCATGCTTGCATACTTCTGCCTGCTGGGAGCCTGG	4620
	GGACTTTCCACACCTGGTGCTGACTAATTGAGATGCATGCTTGCATACTTCTGCCTGC	4680
	TGGGGAGCCTGGGACTTTCCACACCTAACTGACACACATTCCACAGAATTATCCCG	4740
	ATCCCGCTGACCTCGAGAGCTGGCTAATCATGGTCAAGCTGTTCTGTGTGAAATT	4800
	GTTATCCGCTCACAAATTCCACACACATACGAGCCGGAAAGCATAAAGTGTAAAGCCTGG	4860
40	GTGCCTAATGAGTGAACCTCACATTAAATTGCGTTGCGCTCACTGCCGCTTCCAGT	4920
	CGGGAAACCTGCGCAGCTGCATTAATGAATCGCCAACCGCGGGAGAGGCGGTT	4980
	TGGGTATTGGCGCTCTCCGCTTCTCGCTACTGACTCGCTGCGCTCGGTGTTCCGG	5040
	TGCGCGAGCGGTATCAGCTACTAAAGCGGTAAACCGTTATCCACAGAAATCAGGGG	5100
	ATAACGCAGGAAAGAACATGTGAGCAAAAGGCCAGCAACCGTAAAGG	5160
45	CCGCCTTGCTGGCTTCCATAGGCTCCGCCCTGACGAGCATCACAAAAATCGAC	5220
	GCTCAAGTCAGAGGTGGCAACCCGACAGGACTATAAAGATACCAGGCTTCCCGCT	5280
	GAAGCTCCCTGCGCTCTCTGACCTGCGCTTACCGGATACCTGTCCGCT	5340
	TTCTCCCTCGGGAAAGCGTGGCGCTTCTCAATGCTCACGCTGTAGGTATCTCAGTTGG	5400
	TGTAGGTGCTCGCTCAAGCTGGCTGTGACGAACCCCCCGTCAAGCCCACCGCT	5460
50	GCGCTTATCCGTAACTATCGTCTGAGTCAACCCGGTAAGACACGACTATCGCAC	5520
	TGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCAGGTATGAGGCGGTACAGAGT	5580
	TCTTGAAGTGGTGGCTTAACACTACGGCTACACTAGAAGGACAGTATTGGTATCTGCGCTC	5640
	TGCTGAAGCCAGTTACCTTCGGAAAAGAGTGGTAGCTTGTGATCCGGAAACAAACCA	5700
	CCGCTGGTAGCGGTGGTTTTGTTGCAAGCAGCAGATTACGCGCAGAAAAAAGGAT	5760

	CTCAAGAAGATCCTTGTACTTTCTACGGGGTCTGACGCTCAGTGGAACGAAACTCAC	5820
	GTAAAGGGATTTGGTCATGAGATTATCAAAGGATCTCACCTAGATCCTTTAAATT	5880
	AAAAATGAAGTTAAATCAATCTAAAGTATATGAGTAAACTTGGTCTGACAGTTACC	5940
5	AATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTCGTTCATCCATAGTTG	6000
	CCTGACTCCCCGTCGTAGATAACTACGATACGGGAGGGTCTACCATCTGGCCCCAGTG	6060
	CTGCAATGATACCGCGAGACCCACGCTCACCGGCTCCAGATTATCAGCAATAAACCGC	6120
	CAGCCGGAAGGGCGAGCGCAGAAGTGGTCTGCAACTTATCCGCTCCATCCAGTCTA	6180
	TTAATTGTTGCCGGGAAGCTAGAGTAAGTAGTTGCCAGTTAATAGTTGCGAACGTTG	6240
	TTGCCATTGCTACAGGCATCGTGGTGTACGCTCGTCTGGTATGGCTTCATTAGCT	6300
10	CCGGTCTCCAACGATCAAGGCAGGTTACATGATCCCCATGTTGCAAAAAGCGGTTA	6360
	GCTCCTTCGGTCTCCGATCGTTGTCAGAAGTAAGTTGCCGAGTGTATCATCATGG	6420
	TTATGGCAGCACTGCATAATTCTTACTGTCTAGCCATCGTAAGATGCTTCTGTGA	6480
	CTGGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGTATGCCGACCGAGTTGCTCTT	6540
15	GCCCGCGTCAACATACGGGATAATACCGCGCACATAGCAGAACCTTAAAGTGTCTAC	6600
	TTGGAAAACGTTCTCGGGCGAAAACGATCTCAAGGATCTACCGCTGTTGAGATCCAGTT	6660
	CGATGTAACCCACTCGTGCACCCAACTGATCTCAGCATCTTACTTTACCGCTGTTGAGATCCAGTT	6720
	CTGGGTGAGCAAAAACAGGAAGGAAAATGCCGAAAAAAGGGAAATAAGGGCACACGGA	6780
	AATGTTGAATACTCATACTCTCCCTTTCAATATTATTGAAGCATTATCAGGGTTATT	6840
20	GTCTCATGAGCGGATACATATTTGAATGTATTAAGAAAATAACAAATAGGGTTCCGC	6900
	GCACATTCCCCGAAAAGTGCCACCT	6926

Table 3. DNA sequence of IL4.Y124D/IgG1 fusion molecule coding region, 1164bp

25	SEQ ID No:3	
	ATGGGTCTCACCTCCAACTGCTTCCCCCTCTGTTCTTCTGCTAGCATGTGCCGGAAC	60
	TTTGTCCACGGACACAAGTGCATATCACCTACAGGAGATCATCAAACCTTGAACAGC	120
	CTCACAGAGCAGAAGACTCTGTGCACCGAGTTGACCGTAACAGACATCTTGCTGCC	180
	AAGAACACAACGTGAGAAGGAAACCTTCTGCAGGGCTGCGACTGTGCTCCGGAGTTCTAC	240
30	AGCCACCATGAGAAGGACACTCGTGCCTGGGTGCGACTGCACAGCAGTTCCACAGGCAC	300
	AAGCAGCTGATCCGATTCCTGAAACGGCTCGACAGGAACCTCTGGGCTGGGGCTTG	360
	AATTCTGTCTGTGAAGGAAGCCAACCAGAGTACGTTGAAAACCTTCTGAAAGGCTA	420
	AAGACGATCATGAGAGAGAAAGACTCAAAGTGTTCGAGCGGTACCGAGCCAAATCGGCC	480
	GACAAAACATCACACATGCCAACCGTCCCAGCACCTGAACCTCTGGGGGACCGTCAGTC	540
35	TTCCTTCCCCCAAAACCAAGGACACCCCTCATGATCTCCGGACCCCTGAGGTACA	600
	TGGCGTGGGGTGGACGTGAGGCCACGAAGACCCCTGAGGTCAAGTTCAACTGGTACGTGGAC	660
	GGCGTGGAGGTGCATAATGCCAACAGACAAAGCCGGGGAGGAGCAGTACAACAGCACGTAC	720
	CGGGTGGTCAGCGTCTCACCGTCTGCACCCAGGACTGGCTGAATGGCAAGGAGTACAAG	780
40	TGCAAGGTCTCCAACAAAGCCCTCCAGCCCCCATCGAGAAAACCATCTCAAAGCCAA	840
	GGGCAGCCCCGAGAACACAGGGTGTACACCCCTGCCCTCCAGGGATGAGCTGACCAAG	900
	AACCAAGGTGAGCCCTGACCTGCCTGGTCAAAGGCTCTATCCCAGCGACATGCCGTGGAG	960
	TGGGAGAGCAATGGGAGCCGGAGAACAACTACAAGACACGCCCTCCGTGCTGGACTCC	1020
	GACGGCTCTTCTTCCCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGG	1080
45	AACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAACGAGC	1140
	CTCTCCCTGTCTCGGGTAAATGA	1164

Table 4. Sequence of encoded IL4.Y124D/IgG1 fusion protein, 387aa

50 SEQ ID No:4

1	MGLTSQLLPP LFFLLACAGN FVHGHKCDIT LQEIIKTLNS LTEQKTLCTE	
51	LTVTDIFAAAS KNTTEKETFC RAATVLRQFY SHHEKDTRCL GATAQQFHRH	

101 KQLIRFLKRL DRNLWGLAGL NSCPVKEANQ STLENFLERL KTIMREKDSK
 151 CSSGTEPKSA DKHTCPPCP APELLGGPSV FLFPPPKD LMISRTPEWT
 201 CVVVDVSHED PEVKFNWYVD GVEVHNAKTK PREEQYNSTY RVSVLTVLH
 251 QDWLNGKEYK CKVSNKALPA PIEKTISKAK GQPREPQVYT LPPSRDELTK
 5 301 NQVSLTCLVK GFYPSDIAVE WESNGOPENN YKTPPVLDs DGSFFLYSKL
 351 TVDKSRWQQG NVFSCSVMHE ALHNHYTQKS LSLSPGK*

Table 5. DNA sequence of synthetic IgG4 cDNA, 1006bp

10	SEQ ID No:5	
	GCTTCCACCAAGGGCCCATCCGTCTTCCCCCTGGCGCCCTGCTCCAGGAGCACCTCCGAG	60
	AGCACAGCCGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTG	120
	TGGAACTCAGGGCCCTGACCAGCGCGTGCACACCTCCCGGCTGTCTACAGTCCCTCA	180
15	GGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTGGGACGAAGACC	240
	TACACCTGCAACGTAGATACAAGCCCAGCAACACCAAGGGACAAGAGAGTTGAGTCC	300
	AAATATGGTCCCCCATGCCCATATGCCCATGCCCAGCACCTGAATTCTGGGGGACCATCAGTC	360
	TTCTCTTCCCCAAAACCCAAGGACACTCTCATGATCTCCCGGACCCCTGAGGTACG	420
	TGGCTGGTGGTGGACGTGAGCCAGGAAGACCCCAGGGTCAAGTCAACTGGTACGTGGAT	480
20	GGCGTGGAGGTGCATAATGCAAGACAAGGCCGGAGGAGCAGTTCAACAGCACGTAC	540
	CGTGTGGTCAGCGTCCTCACCGTCTGCACCAGGACTGGCTGAACGGCAAGGAGTACAAG	600
	TGCAAGGTCTCCAACAAAGGCCCTCCGTCATCGATCGAGAAAACATCTCAAAGCCAA	660
	GGCAGCCCCGAGAGCCACAGGTGTACACCCCTGCCCTCATCCAGGAGGAGATGACCAAG	720
25	AACCAAGGTCACTGGCAGCCGGAGAACAAACTACAAGACCAAGCCCTCCGTGCTGGACTCC	780
	TGGGAGAGCAATGGCAGCCGGAGAACAAACTACAAGACCAAGCCCTCCGTGCTGGACTCC	840
	GACGGATCTCTTCTCTACAGCAGGTAACCGTGGACAAGAGCAGGTGGCAGGAGGGG	900
	AATGCTTCTCATGCTCCGTGATGCACTGGCTCTGCACAACCAACTACACACAGAAGAGC	960
	CTCTCCCTGTCTGGTAAATGAGTAGTCTAGATCTACGTATG	1006

Table 6. DNA sequence of IL4.Y124D/IgG4 fusion molecule coding region, 1149bp

30	SEQ ID No:6	
	ATGGGTCTCACCTCCAAC TGCTTCCCCCTGTTCTTCTGCTAGCATGTGCCGGCAAC	60
35	TTTGTCCACGGACACAAGTGCATATCACCTTACAGGAGATCATAAAACATTGAACAGC	120
	CTCACAGAGCAGAACACTCTGTGCACCGAGTTGACCGTAACAGACATCTTGCTGCCCTCC	180
	AAGAACACAACGTGAGAAGGAAACCTCTGCAGGGCTGCGACTGTGCTCCGGCAGTTCTAC	240
	AGCCACCATGAGAAGGACACTCGCTGCCTGGTGCAGTCACAGCAGTTCCACAGGCAC	300
	AAGCAGCTGATCCGATTCTCTGAAACGGCTCGACAGGAACCTCTGGGCTGGCGGGCTTG	360
40	AATTCTGTCTGTGAAGGAAGCCAACCAAGAGTACGTTGGAAAATTCTTGAAAGGCTA	420
	AAGACGATCATGAGAGAGAACACTCAAAGTGCAGCGAGTCAAATATGGTCCCCCA	480
	TGCCCATCATGCCAGCACCTGAATTCTGGGGGACCATCAGTCTTCTGTTCCCCCA	540
	AAACCCAAGGACACTCTCATGATCTCCCGGACCCCTGAGGTCACTGCGTGGTGGAC	600
	GTGAGCCAGGAAGACCCCAGGTCAAGTCAACTGGTACGTGGATGGCGTGGAGGTGCAT	660
45	AATGCCAAGACAAGCCGGGGAGGAGCAGTTAACAGCACGTACCGTGTGGTCAGCGTC	720
	CTCACCGTCTGCACCAGGACTGGCTGAACGGCAAGGAGTACAAGTGCAGGTCTCCAAC	780
	AAAGGCCTCCCGTCATCGATCGAGAAAACATCTCAAAGCCAAGGGCAGCCCCGAGAG	840
	CCACAGGTGACACCCCTGCCCTCATCCAGGAGGAGATGACCAAGAACCAAGGTCAAGCCTG	900
	ACCTGCCTGGTCAAAGGTTCTACCCCAAGCGACATCGCCCTGGAGTGGAGAGCAATGGG	960
50	CAGCCGGAGAACAACTACAAGACCAAGCCCTCCGTGCTGGACTCCGACGGATCTTCTTC	1020
	CTCTACAGCAGGCTAACCGTGGACAAGAGCAGGTGGCAGGGAGGGAAATGTCTCTCATGC	1080
	TCCGTGATGCACTGGCTCTGCACAACCAACTACACACAGAAGAGCCTCTCCCTGTCTG	1140
	GGTAAATGA	1149

Table 7. Sequence of encoded IL4.Y124D/IgG4 fusion protein, 382aa

5 SEQ ID No:7

1	MGLTSQLLPP LFFLLACAGN FVHGHKCDIT LQEIIKTLNS LTEQKTLCTE
51	LTVDIFAAKS KNTTEKETFC RAATVLRQFY SHHEKDTRCL GATAQQFHRH
101	KQLIRFLKRL DRNLWGLAGL NSCPVKEANQ STLENFLERL KTIMREKDSK
151	CSSESKYGPP CPSCPAPAEFL GGPSVFLFP KPDKTLMISR TPEVTCVVVD
201	VSQEDPEVQF NWYVDGVEVH NAKTKPREEQ FNSTYRVVSV LTVLHQDWLN
251	GKEYKCKVSN KGLPSSIEKT ISKAKGQPREG QPVYTLPPSQ EEMTKNQVSL
301	TCLVKGFYPS DIAVEWESNG QPENNYKTTTP PVLDSDGSFF LYSRLTVDKS
351	RWQEGNVFSC SVMHEALHNH YTQKSLSSL GK*

15 Table 8. DNA sequence of IgG4 PE variant, 984bp

SEQ ID No:8

1	GCTAGTACCAAGGGCCATCCGTCTCCCCCTGGGCCCTGCTCCAGGAGCACCTCCGAG	60
20	AGCACGCGCCCTGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTG 120	
	TGGAACTCAGGCAGCCCTGACCAGCGCGTGCACACCTTCCCCTGCTCCTACAGTCCTCA	
	GGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCTCCAGCAGCTGGCACGAAGACC 180	
	TACACCTGCAACGTAGATCACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGTCC 240	
	AAATATGGTCCCCCATGCCCacCATGCCAGCgCCTGAA TTtgaGGGGGACCATCAGTC 300	
25	TTCCGTCTCCCCCAAACCCAAGGACACTCTCATGATCTCCGGACCCCTGAGGTACAG 360	
	TGCGTGGTGGTGGACGTGAGCCAGGAAGACCCGAGGTCCAGTTCAACTGGTACGTGGAT 420	
	GGCGTGGAGGTGATAATGCCAACAGACAAAGCCGCGGGAGGAGCAGTTCAACAGCACGTAC 480	
	CGTGTGGTCAGCGTCTCACCGTCTGCACCAGGACTGGCTGAACGGCAAGGAGTACAAG 540	
30	TGCAAGGTCTCAACAAAGCCTCCGTCA TGcATCGAGAAAACCATCTCAAAGCCAA 600	
	GGGCAGCCCCGAGAGCCACAGGTGTACACCTCTGCCCTCATCCCAGGAGGAGATGACCAAG 660	
	AACCAGGTCACTGCCTGACCTGCCTGGTCAAAGGTTCTACCCAGCGACATGCCGTGGAG 720	
	TGGGAGAGCAATGGGAGCCGGAGAACAAACTACAAGACCAAGCGCCTCCGTGCTGGACTCC 780	
	GACGGATCCTCTTCTCTACAGCAGGCTAACGTGGACAAGAGCAGGTGGCAGGAGGGG 840	
35	AATGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCAACTACACACAGAAGAGC 900	
	CTCTCCCTGTCTCTGGTAAATGA 960	
		984

Table 9. DNA sequence of IL4.Y124D/IgG4 PE fusion molecule coding region, 1149bp

40 SEQ ID No:9

1	ATGGGTCTCACCTCCAACTGCTCCCCCTCTGTTCTCTGCTAGCATGTGCCGGCAAC	60
	TTTGTCCACGGACACAAGTGCATATCACCTTACAGGAGATCATCAAAACTTGAACAGC	
45	CTCACAGAGCAGAAGACTCTGTGACCGAGTTGACCGTAACAGACATCTTGCTGCCCTC 120	
	AAGAACACAACGTAGAGAGAACCTTCTGCAGGGCTGCGACTGTGCTCCGGCAGTCTAC	
	AGCCACCATGAGAAGGACACTCGCTGCCCTGGGTGCGACTGCACAGCAGTTCACAGGCAC 180	
	AAGCAGCTGATCCGATTCTGAAACGGCTCGACAGGAACCTCTGGGGCTGGGGCTTG 240	
50	AATTCCGTCTGTGAAGGAAGCAACCAGAGTACGTGGAAAACCTCTTGGAAAGGCTA 300	
	AAGACGATCATGAGAGAGAACACTCAAAGTGCAGCGAGTCCAAATATGGTCCCCCA 360	
	TGCCACCATGCCAGCgCCTGAATTGAGGGGGACCATCAGTCTCTGTTCCCCCA 420	
	AAACCCAAGGACACTCTCATGATCTCCGGACCCCTGAGGTACGTGGATGGCGTGGAGGTGCAT 480	
	GTGAGCCAGGAAGACCCGAGGTCCAGTTCAACTGGTACGTGGATGGCGTGGAGGTGCAT 540	
	AATGCCAAGACAAAGCCGGGGAGGAGCAGTTCAACAGCACGTACCGTGTGGTCAGCGTC 600	
		660
		720

	CTCACCGTCCCTGCACCAGGACTGGCTAACGGCAAGGAGTACAAGTGCAAGGTCTCCAAC	780
	AAAGGCCCTCCCGTCatCgATCGAGAAAACCATCTCCAAAGCCAAGGGCAGCCCCGAGAG	840
	CCACAGGTGTACACCCTGCCCCCATCCCAGGAGGAGATGACCAAGAACCAAGGTCAAGCTG	900
	ACCTGCCTGGTCAAAGGCTTCTACCCAGCGACATGCCGTGGAGTGGAGAGCAATGGG	960
5	CAGCCGGAGAACAACTACAAGACCACGCCCTCCCGTGTGGACTCCGACGGaTCCTTCTTC	1020
	CTCTACAGCAGGCTAACCGTGGACAAGAGCAGGTGGCAGGAGGGGAATGTCTTCATGC	1080
	TCCGTGATGCATGAGGCTCTGCACAACCAACTACACACAGAAGAGCCTCTCCGTCTCG	1140
	GGTAAATGA	1149

10

Table 10. Sequence of encoded IL4.Y124D/IgG4 PE variant fusion protein, 382aa

SEQ ID No:10

15	1 MGLTSQLLPP LFFLLACAGN FVHGHKCDIT LQEIIKTLNS LTEQKTLCTE	
	51 LTVTDIFAAS KNTTEKETFC RAATVLRQFY SHHEKDTRCL GATAQQFHRH	
	101 KQLIRFLKRL DRNLWGLAGL NSCPVKEANQ STLENFLERL KTIMREKDSK	
	151 CSSESKYGPP CPPCPAPEFE GGPSVFLFFF KPDKTLMISR TPEVTCVVVD	
	201 VSQEDPEVQF NWYVDGVEVH NAKTKPREEQ FNSTYRVVSV LTVLHQDWLN	
	251 GKEYKCKVSN KGLPSSIEKT ISKAKGQPRES PQVYTLPPSQ EEMTKNQVSL	
20	301 TCLVKGFYPS DIAVEWESNG QPENNYKTP PVLDSDGSFF LYSRLTVDKS	
	351 RWQEGNVFSC SVMHEALHNH YTQKSLSLSL GK*	

CLAIMS

1. A soluble protein having IL4 and/or IL13 antagonist or partial antagonist activity,
5 comprising an IL4 mutant or variant fused to least one human immunoglobulin constant domain or fragment thereof.
2. A compound according to claim 1, wherein at least one amino acid, naturally occurring in wild type IL4 at any one of positions 120 to 128 inclusive, is replaced by a different
10 natural amino acid.
3. A compound according to claim 2, wherein the tyrosine naturally occurring at position 124 is replaced by a different natural amino acid.
- 15 4. A compound according to claim 3, wherein the tyrosine naturally occurring at position 124 is replaced by aspartic acid.
5. A compound according to any one of the preceding claims, wherein the immunoglobulin is of the IgG subclass
20
6. A compound according to claim 5, wherein the constant domain(s) or fragment thereof is the whole or a substantial part of the constant region of the heavy chain of human IgG.
- 25 7. A compound according to claim 5, wherein the constant domain(s) or fragment thereof is the whole or a substantial part of the constant region of the heavy chain of human IgG4.
8. A compound according to claim 1, having the amino acid sequence represented by
30 SEQ ID No:4, SEQ ID No:7 or SEQ ID No:10.
9. A process for preparing a compound according to any one of the preceding claims, which process comprises expressing DNA encoding said compound in a recombinant host cell and recovering the product.

10. A process according to claim 9, which comprises:
 - i) preparing a replicable expression vector capable, in a host cell, of expressing a DNA polymer comprising a nucleotide sequence that encodes said compound;
 - ii) transforming a host cell with said vector;
 - 5 iii) culturing said transformed host cell under conditions permitting expression of said DNA polymer to produce said compound; and
 - iv) recovering said compound.
11. A DNA polymer comprising a nucleotide sequence that encodes a compound
10 according to any one of claims 1 to 8.
12. A DNA polymer according to claim 11, which comprises or consists of the sequence of SEQ ID No:3, SEQ ID No:6 or SEQ ID No:9.
15 13. A replicable expression vector comprising a DNA polymer according to claim 11.
14. A host cell transformed with a replicable expression vector according to claim 13.
15 15. A pharmaceutical composition comprising a compound according to any one of
20 claims 1 to 8 and a pharmaceutically acceptable carrier.
16. A method of treating conditions resulting from undesirable actions of IL4 and/or IL13 which comprises administering to the sufferer an effective amount of a compound according to claim 1.
25 17. A compound according to any one of claims 1 to 8, for use in therapy.
18. A compound according to any one of claims 1 to 8, for use in the treatment of conditions resulting from undesirable actions of IL4 and/or IL13.
30 19. Use of a compound according to any one of claims 1 to 8 in the manufacture of a medicament for use in the treatment of conditions resulting from undesirable actions of IL4 and/or IL13.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 95/03036

A. CLASSIFICATION OF SUBJECT MATTER	IPC 6 C12N15/62	C07K14/54	C07K16/00	C07K19/00	A61K38/19
	A61K39/395				

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EP,A,0 464 533 (BEHRINGWERKE) 8 January 1992 cited in the application see claims; examples ---	1-7,10, 11,13-19
Y	WO,A,93 10235 (SEBALD) 27 May 1993 cited in the application see the whole document ---	1-7,10, 11,13-19
Y	EMBO JOURNAL, vol. 12, no. 7, July 1993 EYNSHAM, OXFORD GB, pages 2663-2670, S.M. ZURAWSKI ET AL 'Receptors for Interleukin-13 and interleukin-4 are complex and share a novel component that functions in signal transduction' see the whole document ---	1-7,10, 11,13-19 -/-

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

* Special categories of cited documents:

- *'A' document defining the general state of the art which is not considered to be of particular relevance
- *'E' earlier document but published on or after the international filing date
- *'L' document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *'O' document referring to an oral disclosure, use, exhibition or other means
- *'P' document published prior to the international filing date but later than the priority date claimed

- *'T' later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *'X' document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *'Y' document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- *'&' document member of the same patent family

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Date of the actual completion of the international search

12 December 1995

Date of mailing of the international search report

03.01.96

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INTERNATIONAL SEARCH REPORT

Inteⁿl Application No
PCT/EP 95/03036

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	MOLECULAR IMMUNOLOGY, vol. 30, no. 1, January 1993 pages 105-108, S. ANGAL ET AL 'A singgle amino acid sustitution abolishes the heterogeneity of chimeric mouse/human (IgG4) antibody' cited in the application see the whole document ---	1-7, 10, 11, 13-19
Y	WO,A,88 07089 (MEDICAL RESEARCH COUNCIL) 22 September 1988 see the whole document & EP,A,0 307 434 (MEDICAL RESEARCH COUNCIL) cited in the application ----	1-7, 10, 11, 13-19
A	EP,A,0 367 166 (TAKEDA CHEMICAL INDUSTRIES. LTD.) 9 May 1990 see claims -----	1

INTERNATIONAL SEARCH REPORT

International application No.

PCT/EP 95/03036

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.: **16**
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although this claim is directed to a method of treatment of the human/animal body (Rule 39.1(iv)), the search has been carried out and based on the alleged effects of the compound/composition.
2. Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- The additional search fees were accompanied by the applicant's protest.
- No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No.

PCT/EP 95/03036

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A-464533	08-01-92	AU-B-	655421	22-12-94
		AU-B-	7935791	02-01-92
		CA-A-	2045869	29-12-91
		JP-A-	5247094	24-09-93
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		AU-A-	2928292	15-06-93
		CA-A-	2123315	27-05-93
		CZ-A-	9401185	15-12-94
		EP-A-	0613499	07-09-94
		HU-A-	66826	30-01-95
		JP-T-	7501522	16-02-95
		NO-A-	941681	06-05-94
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		AU-B-	1480388	10-10-88
		DE-D-	3883899	14-10-93
		DE-T-	3883899	31-03-94
		EP-A,B	0307434	22-03-89
		EP-A-	0351410	24-01-90
		WO-A-	8807054	22-09-88
		GB-A,B	2209757	24-05-89
		JP-T-	1502875	05-10-89
EP-A-367166	09-05-90	AU-B-	622724	16-04-92
		AU-B-	4391089	03-05-90
		JP-A-	2209898	21-08-90